



In re Application of: Akira ASAKURA, *et al.*
Serial No.: 09/470,667
For: **NOVEL ALCOHOL/ALDEHYDE
DEHYDROGENASES**

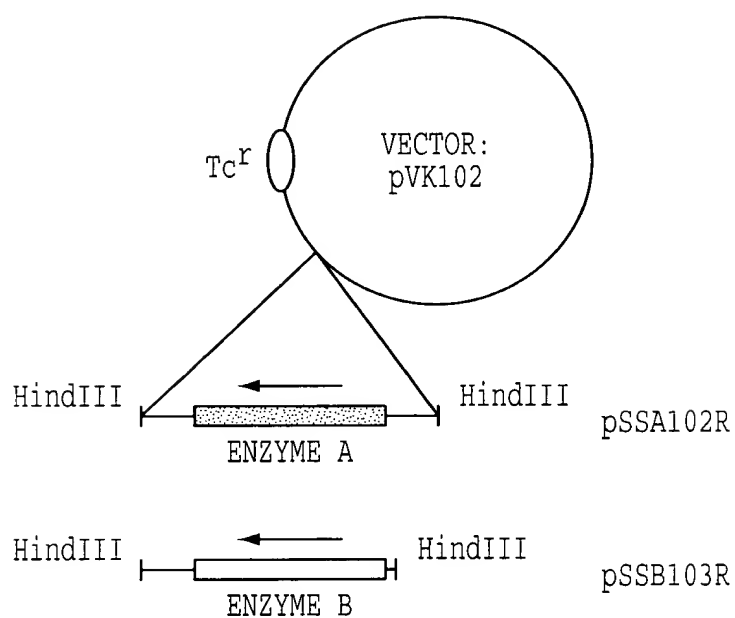


FIG. 1



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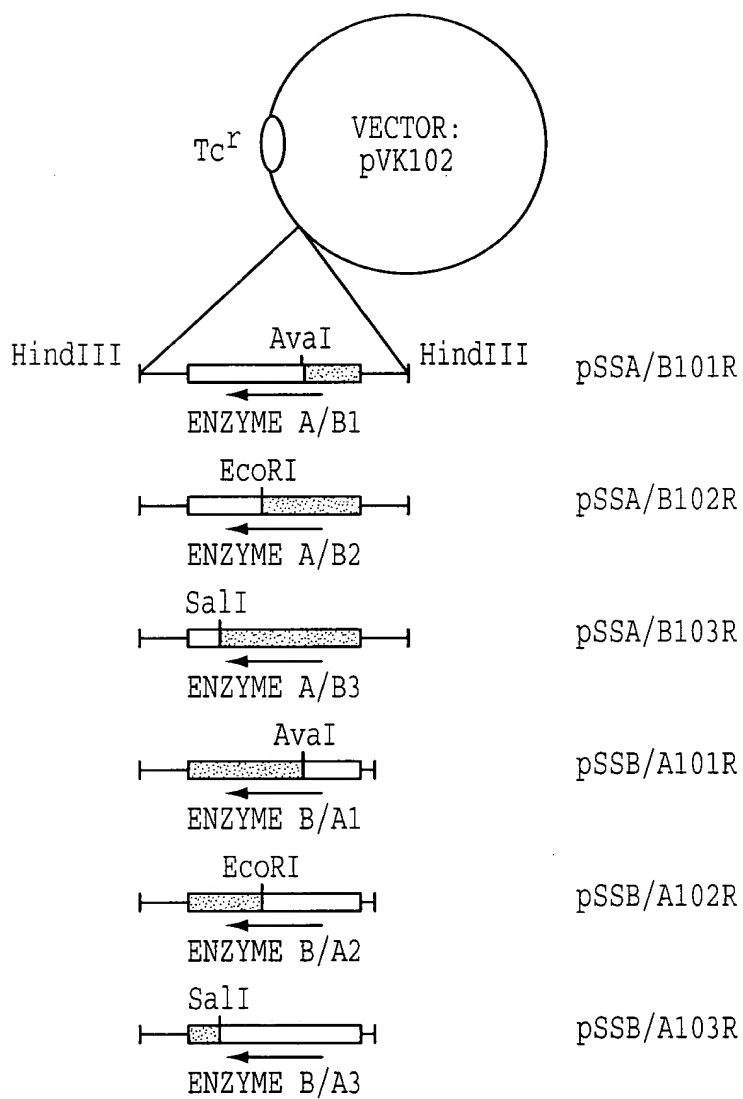
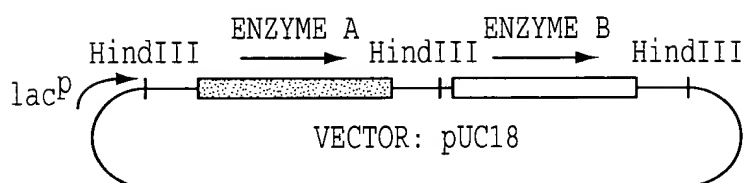


FIG. 2



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pSSAB201



pSSBA201

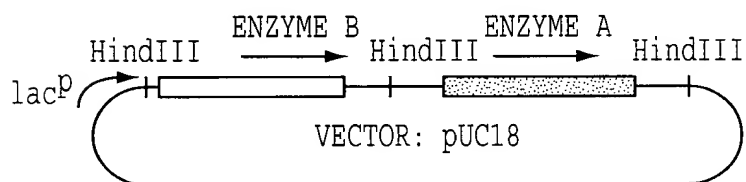
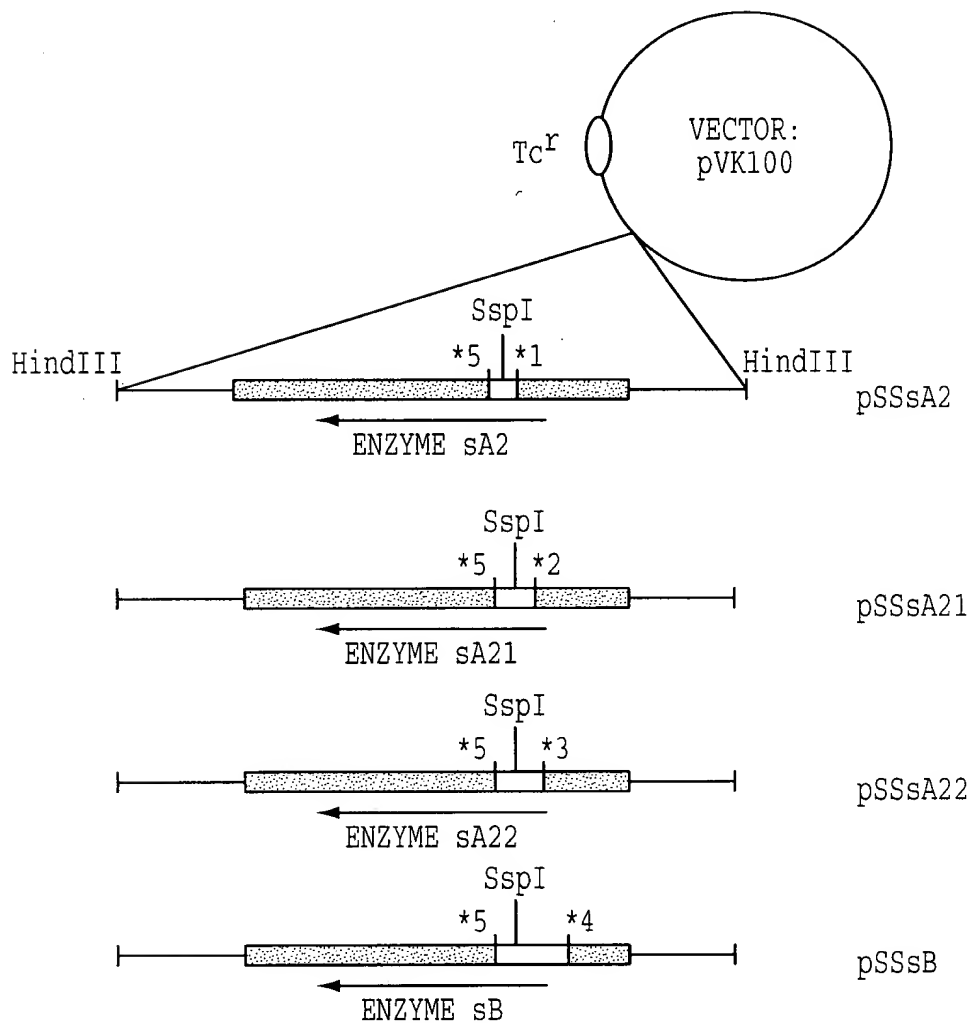


FIG. 3



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RECOMBINATION SITE

- *1 : AMINO ACID RESIDUE NO. 135 OF MATURE ENZYME A
- *2 : AMINO ACID RESIDUE NO. 128 OF MATURE ENZYME A
- *3 : AMINO ACID RESIDUE NO. 125 OF MATURE ENZYME A
- *4 : AMINO ACID RESIDUE NO. 95 OF MATURE ENZYME A
- *5 : AMINO ACID RESIDUE NO. 180 OF MATURE ENZYME B,
 WHICH NUCLEOTIDE SEQUENCE OF *Ava*I SITE ENCODES

FIG. 4



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ENZYME A 1 : QVTPVTDELL ANPPAGEWIS YGQNQENYRH SPLTQITTEN VGQLQLVWAR GMQPGKVQVT

ENZYME B 1 : QVTPITDELL ANPPAGEWIN YGRNQENYRH SPLTQITADN VGQLQLVWAR GMEAGAVQVT

61 : PLIHGVMYL ANPGDVIQAI DAKTGDLIWE HRRQLPNIAI LNSFGEPTRG MALYGTNVYF
*
61 : PMIHGVMYL ANPGDVIQAL DAQTGDLIWE HRRQLPAVAT LNAQGDRKRG VALYGTSLYF

AvaI

121 : VSWDNHLVAL DTATGQVTFD VDRGQGED-M VSNSSGPIVA NGVIVAGSTC QYSPFGCFVS

121 : SSWDNHLIAL DMETGQVVFD VERGSGEDGL TSNTTGPIVA NGVIVAGSTC QYSPYGC FIS

180 : GHDSATGEEL WRNYFIPRAG EEGDETWGND YEARWMTGAW GQITYDPVTN LVHYGSTAVG

181 : GHDSATGEEL WRNHFIPQPG EEGDETWGND FEARWMTGVW GQITYDPVTN LVFYGSTGVG

240 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVTNVDVQPS

241 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVANVDVQPS

EcoRI

300 : TEMEGLQSIN PNAATGERRV LTGVPCKTGT MWQFDAETGE FLWARDTNYQ NMIESIDENG

301 : AEMEGLRAIN PNAATGERRV LTGAPCKTGT MWSFDAASGE FLWARDTNYT NMIASIDETG

360 : IVTVNEDAIL KELDVEYDVC PTFLGGRDWP SAALNPDSGI YFIPLNNVCY DMMAVDQEFT

361 : LVTVNEDAVL KELDVEYDVC PTFLGGRDWS SAALNPDTGI YFLPLNNACY DIMAVDQEFS

Sali

420 : SMDVYNTSNV TKLPPGKDMI GRIDAIDIST GRTLWSVERA AANYSPVLST GGGVLFNGGT

421 : ALDVYNTSAT AKLAPGFENM GRIDAIDIST GRTLWSAERP AANYSPVLST AGGVVFNGGT

480 : DRYFRALSQE TGETLWQTRL ATVASGQAIS YEVDGMQYVA IAGGGVSYGS GLNSALAGER

481 : DRYFRALSQE TGETLWQARL ATVATGQAIS YELDGVQYIA IGAGGLTYGT QLNAPLA-EA

540 : VDSTAIGNAV YVFALPQ

540 : IDSTSVGNAI YVFALPQ

* : NUCLEOTIDE SEQUENCES ENCODING THESE REGIONS ARE THE RESTRICTION SITES
FOR AvaI, EcoRI, AND Sali WHICH WERE USED FOR CONSTRUCTING CHIMERA
GENES SHOWN IN FIG. 2.

FIG. 5



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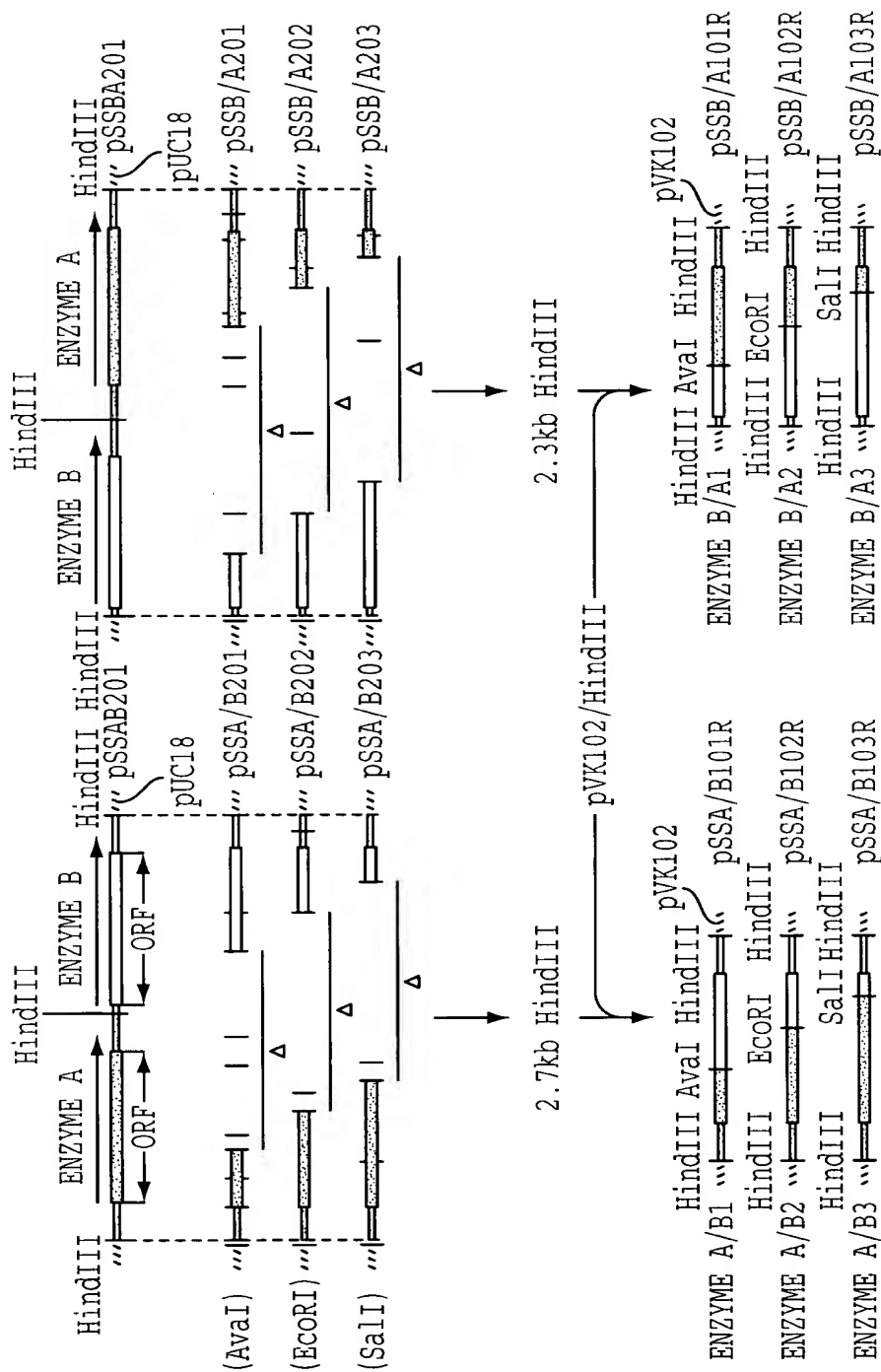
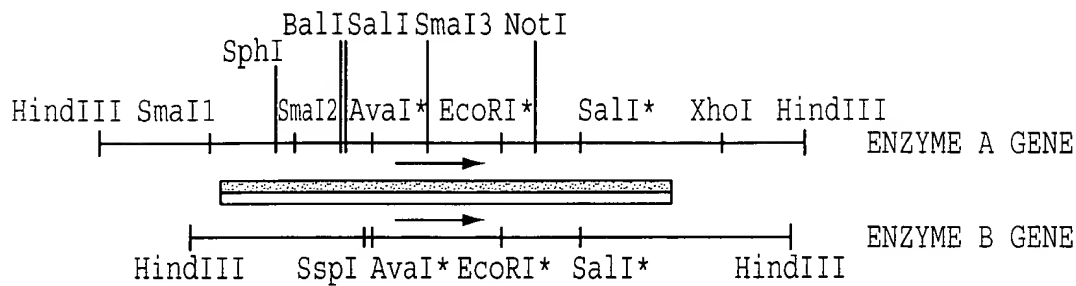


FIG. 6

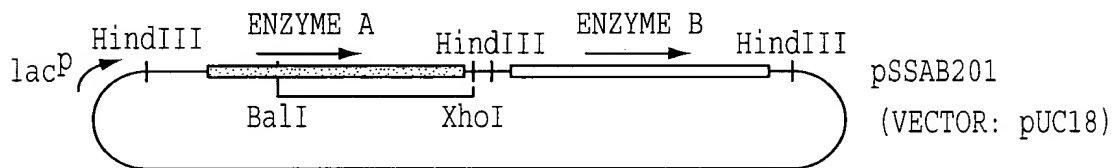


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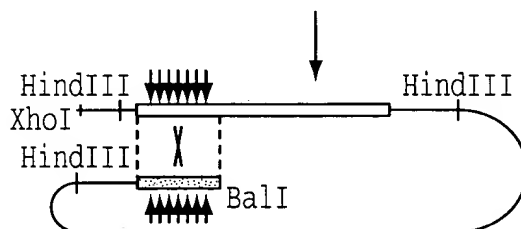


*: AvaI, EcoRI, SalI SITES USED FOR CONSTRUCTING CHIMERA GENES SHOWN IN FIG. 2 AND 6.

FIG. 7



LINEARIZATION WITH XhoI AND BalI



TRANSFORM *E. coli* JM101 (rec A+)

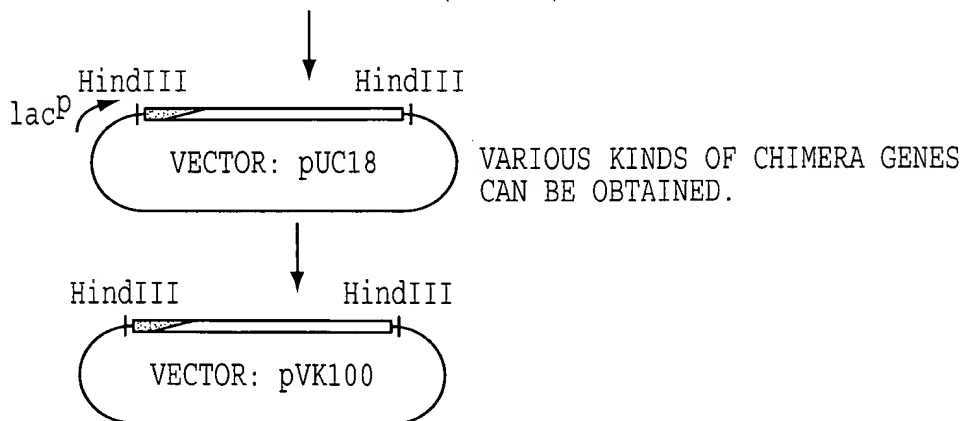


FIG. 8



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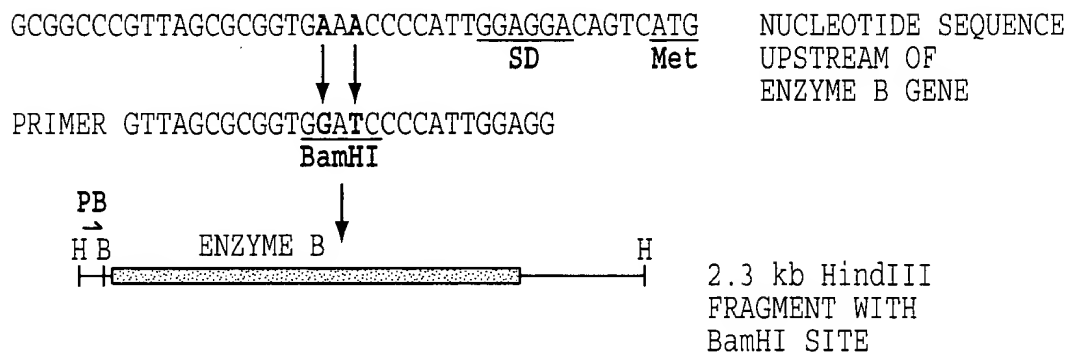


FIG. 9

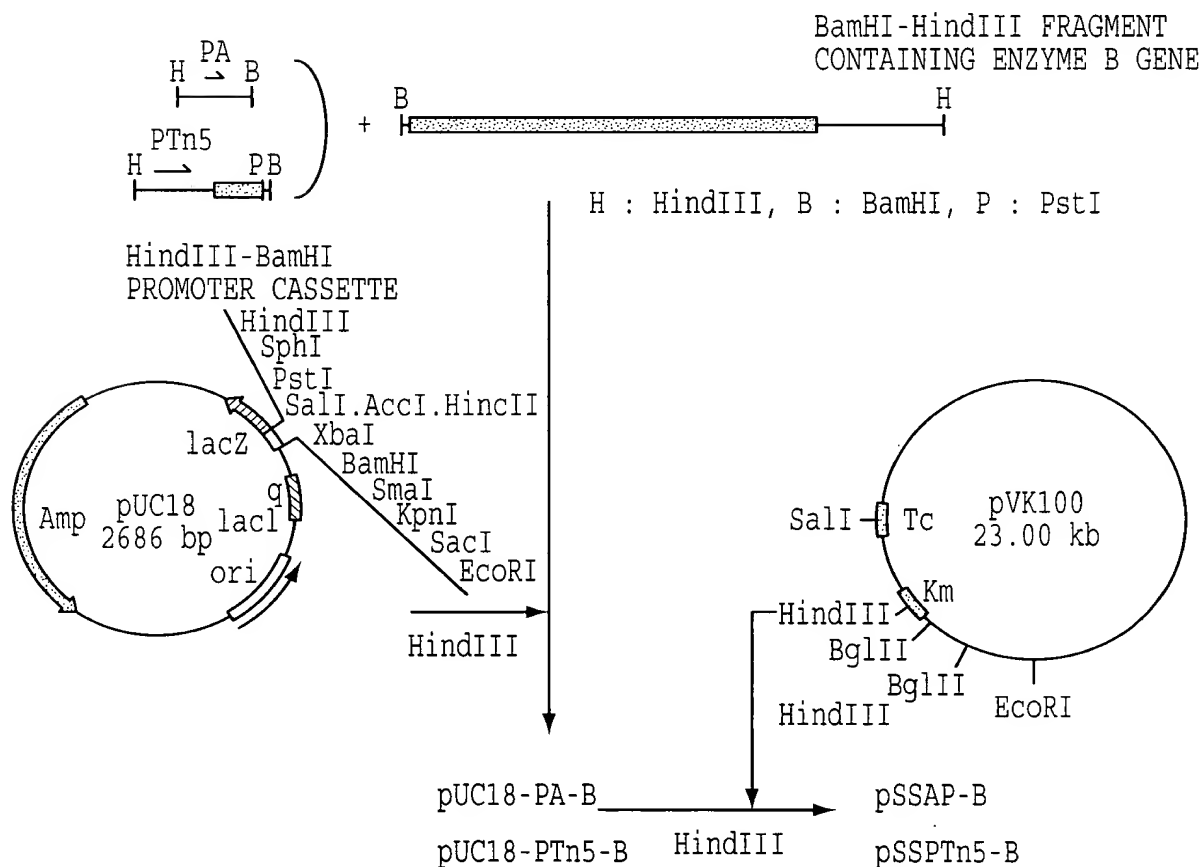
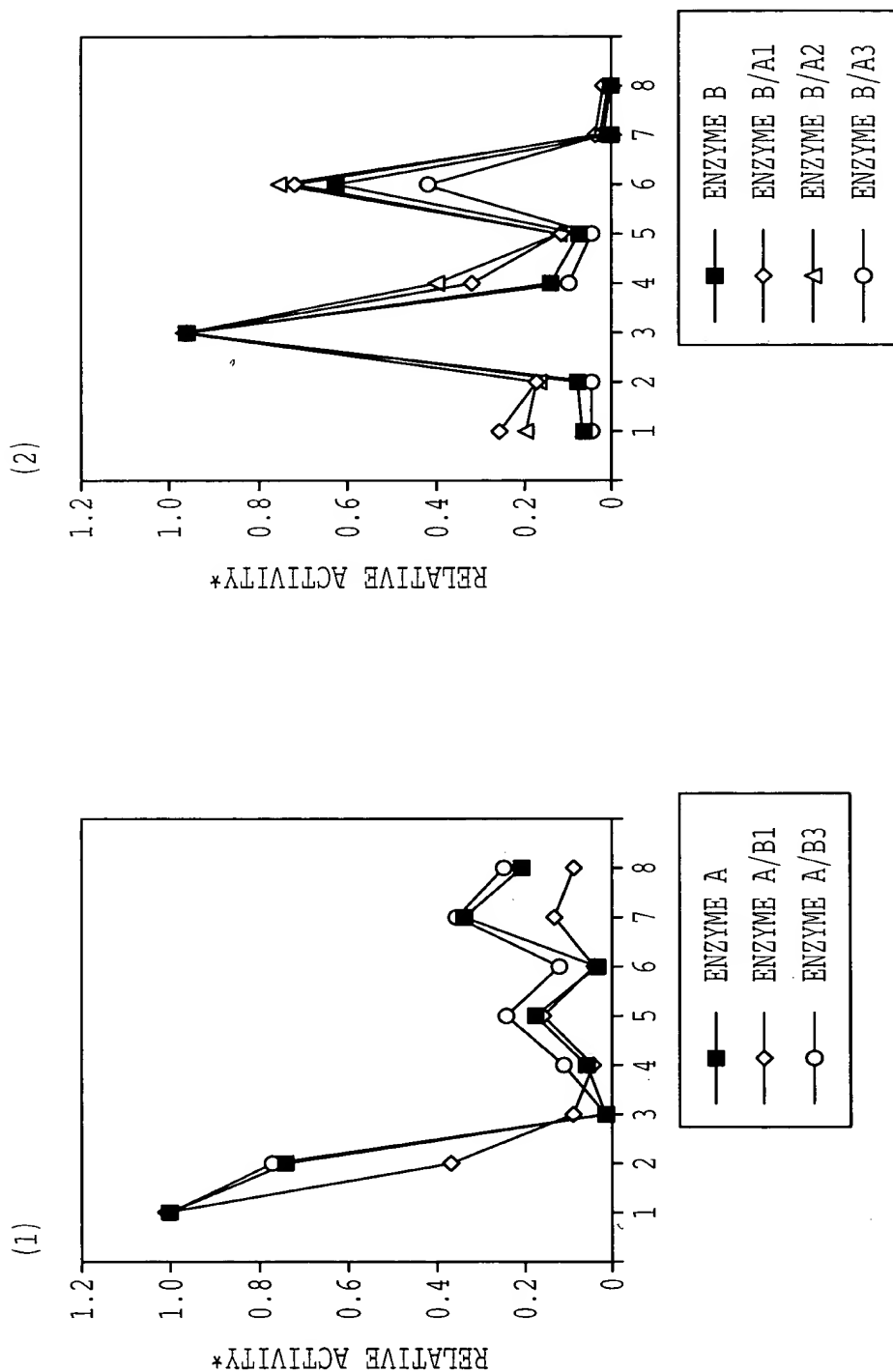


FIG. 10



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1. n-PROPANOL, 2. ISOPROPANOL, 3. D-GLUCOSE, 4. L-SORBOSE
5. D-SORBITOL, 6. D-MANNITOL, 7. L-SORBOSE, 8. D-FRUCTOSE

*ENZYME ACTIVITY WAS NORMALIZED RELATIVE TO ACTIVITY FOR n-PROPANOL (1), OR D-GLUCOSE (2).
ENZYME A/B2 WAS EXCEPTED BECAUSE OF ITS LOW EXPRESSION IN *P. putida*.

FIG. 11